Sagittula stellata gen. nov., sp. nov., a Lignin-Transforming Bacterium from a Coastal Environment

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A numerically important member of marine enrichment cultures prepared with lignin-rich, pulp mill effluent was isolated. This bacterium was gram negative and rod shaped, did not form spores, and was strictly aerobic. The surfaces of its cells were covered by blebs or vesicles and polysaccharide fibrils. Each cell also had a holdfast structure at one pole. The cells formed rosettes and aggregates. During growth in the presence of lignocellulosic or cellulose particles, cells attached to the surfaces of the particles. The bacterium utilized a variety of monosaccharides, disaccharides, amino acids, and volatile fatty acids for growth. It hydrolyzed cellulose, and synthetic lignin preparations were partially solubilized and mineralized. As determined by 16S rRNA analysis, the isolate was a member of the α subclass of the phylum Proteobacteria and was related to the genus Roseobacter. A signature secondary structure of the 16S rRNA is proposed. The guanine-plus-cytosine content of the genomic DNA was 65.0 mol%. On the basis of the results of 16S rRNA sequence and phenotypic characterizations, the isolate was sufficiently different to consider it a member of a new genus. Thus, a novel genus and species, Sagittula stellata, are proposed; the type strain is E-37 (= ATCC 700073).

Lignin degradation in salt marsh ecosystems is an important biogeochemical process due to the high primary productivity in such ecosystems and the abundance of vascular-plant-derived lignocellulosic material (4, 24). While both bacteria and fungi can be involved in the degradation of lignin (54), in aquatic environments bacteria are probably responsible for the utilization of the most refractory components (27, 35). In a salt marsh, bacteria mediate most of the lignin degradation (5). Although members of at least one genus of bacteria known to be involved in lignin degradation in soils (the genus Streptomyces) have been identified in salt marsh sediments (37), little else is known about the identities of the bacterial lignin degraders in these systems. Thus, the isolation of lignolytic bacterial strains from salt marshes is significant from an ecological perspective. In addition, waste from the pulp and paper industry is another important source of polymeric and highly recalcitrant lignin in some coastal marine environments. Microbial communities are known to mineralize this waste in salt marsh ecosystems (33).

In order to study the lignolytic potential of marine organisms, bacteria were isolated from a community growing in seawater with the high-molecular-weight fraction of pulp mill effluent as the sole carbon source. One isolate, strain E-37, became a dominant member of this enrichment community and eventually contributed up to 32% of the community DNA (22). The phenotypic and phylogenetic characteristics of this isolate indicate that it belongs to a new genus of marine bacteria. In this paper we propose the name Sagittula stellata gen. nov., sp. nov., and designate strain E-37 the type strain of this species.

MATERIALS AND METHODS

Isolation. Strain E-37 was isolated on YTSS medium (see below) from an enrichment community culture containing the high-molecular-weight fraction of pulp mill effluent as the sole carbon source (21). The original source was seawater from the coast of Georgia.

Media and culture conditions. YTSS contained 4 g of yeast extract (Difco Laboratories, Detroit, Mich.), 2.5 g of tryptone (Difco), 20 g of sea salts (Sigma Chemical Co., St. Louis, Mo.), 18 g of agar, and 1 liter of distilled water. Other media used were marine agar 2216, marine broth 2216 (Difco), and marine salts basal medium (BM) (2) supplemented with various carbon sources (21). Unless specified otherwise, each carbon source was added at a concentration of 0.1% (wt/vol or vol/vol). BM agar was prepared by adding 18 g of agar per liter. Liquid cultures were grown at 25°C in the dark at a rotatory shaker at 300 rpm. Plate cultures were grown at room temperature.

Morphological, biochemical, and physiological tests. Routine tests, including tests for growth on different carbon sources, bacteriochlorophyll a production, poly-β-hydroxybutyrate accumulation, and other characteristics, were performed as described previously (21). Spore production was determined in BM containing yeast extract at the late exponential phase by heating the culture medium at 80°C for 10 min, after which the culture was serially diluted in the same medium and incubated at room temperature. Endospore formation was also examined by using the Schaeffer-Fulton staining method (38) with Bacillus subtilis as a positive control. Sulphite oxidation was tested in cell extracts by using ferricyanide as an artificial electron acceptor (26). The cells used for this experiment were grown in BM containing acetate, and a solution of filter-sterilized Na2SO3 was added to a final concentration of 20 mM when the cells were still in the exponential phase. Cells of an isolate very closely related to Sulfitobacter pontiacus were used as a positive control. The 16S rRNA sequence of this isolate, strain EE-36 (22), exhibited 97.7% similarity to the ribosomal RNA (rDNA) sequence of Sulfitobacter pontiacus CHLG 10. The use of Na2SO3 as an electron donor was also tested in BM containing decreasing concentrations of acetate (from 20 to 2 mM) and increasing concentrations of Na2SO3 (from 2 mM to 20 mM). The turbidity of each culture at 540 nm was compared with the turbidity of the control (medium without Na2SO3).

Attachment to particles was studied in BM supplemented with different carbon sources and 0.1% lignocellulose powder, birchwood xylan (Sigma), cellulose powder (ICN Biomedicals, Inc., Costa Mesa, Calif.), or glass beads. Lignocellulose was extracted from the salt marsh cord grass Spartina alterniflora as described by Benner et al. (3).

Sequencing of 16S rDNA. The following oligonucleotide primers were used for sequencing (Escherichia coli numbering system): 19F (CTGGCTTGCACGAR CAAACG) (34); 68F (TNANACATGCAAGTCGAKCG); 338F (ACTCCTACGGGAGGCAGCG); 339F (GCTGGCTTGCACGAR CGAACC) (338R; GCTGCCCTCCGATTGGGATTG) (50); 489R (CGGGG GTTTCCTTTTACCA) (MALF-1; see below); 536R (GWATTCCGCGGCCGT CTO) (31); 785F (GGATAGTAGCTCCCGNTGTA) (9); 928R (CCTCTAAC TTATTTGGGTT) (928F [FAACTCCAAAGGAAATTTGACG]); 1408R (ACGCG CGGTGTTGTRC) (31); and 1523R (AGGAGGATCAGCAGCG) (K is G or T, R is A or G, W is A or T, M is A or C, and N is any base).

Comparative analysis of 16S rDNA. For the phylogenetic analyses, only unambiguous positions were considered (positions 60 to 1373; positions 180 to 480 when the sequences 375W-1 and 375W-2 were used [E. coli numbering system]). Parsimony and bootstrap analyses were performed as described by González et al. (21), except that the branch-and-bound method in addition to the heuristic method was used for parsimony analysis. The least-squares method of Hitch and Margoshis (15) contained in the PHYLIP package (14) was also used. Different ingroups and outgroups were used for comparison. Secondary structures were

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predicted with the help of the computer program MulFold (25, 26, 56) and were further refined by eye.

Recovery of related sequences in seawater. The recovery of 16S rDNA sequences from seawater DNA that were related to the E-37T sequence has been described by Gonzalez et al. (22). DNA was extracted from the bacterial community in the seawater that served as the original inoculum for the enrichment cultures. PCR amplification of DNA was carried out with two PCR primers, one designed specifically to target strain E-37T (ROS137-37, 5'-TTCTGTGAGGAGGATGCCC; positions 137 to 154 [E. coli numbering]) and one designed to target a larger group of marine bacteria, including E-37T (MALF-1; 5'-GCGCCTGGGTTCTCCATTACGAGG; positions 486 to 503), generated DNA fragment 37SW-1. A second PCR amplification from seawater DNA, in which we used primer ROS137-37 and a new primer designed to exclude the first sequence obtained (MALF-2; 5'-GCCGGTTCTCCATTACGAGG; positions 486 to 503), generated DNA fragment 37SW-2.

Electron microscopy. The cells used for electron microscopy were grown in BM containing 0.2% glucose and 0.01% yeast extract. Negative staining was performed as described by Gonzalez et al. (21).

DNA base composition. DNA base composition was determined by high-performance liquid chromatography (21, 36).

Cell membrane fatty acid analysis. A fatty acid methyl ester analysis was performed by workers at Microbial ID, Inc. (Newark, Del.) as described previously (21). Strain E-37T was grown on tryptic soy broth (Difco), on which it grew weakly.

Synthetic lignin mineralization. The lignin-degrading ability of E-37T was determined in BM containing 0.2% glucose and 0.01% yeast extract. The amount of radioactively labeled β-carbon or ring carbon dehydrogenase (DHP) of coniferyl alcohol added to the medium was 4,000 dpm/ml. The specific activities of [β-14C]DHP and [ring-14C]DHP were 286,000 and 434,000 dpm/mg, respectively. Radioactively labeled DHP was prepared by R. Trojanowski (University of Gottingen, Gottingen, Germany) who used the method described by Freudenstein and Neish (17). The average molecular weight of DHP was 2,330, as determined by gel permeation chromatography performed with a Sephadex G-50 gel (52). Mineralization was measured in triplicate in 30-ml portions of medium in 125-ml milk dilution bottles; uninoculated bottles were included as controls. After the bottles were opened and the vials were removed, a 1-ml portion of the trapping solution was added directly to 10 ml of ScintiSafe Econo 2 (Fisher Scientific, Pittsburgh, Pa.) scintillation cocktail for counting. The bottles were left open for 5 min before new NaOH solution was added and the bottles were rescaled.

After 30 days of incubation, the amount of soluble lignin in the culture medium was measured. The medium was first flushed with humid air to drive off any remaining 14CO2 and then filtered through 0.22-μm-pore-size filters (Acrodisc Gelman Sciences, Inc., Ann Arbor, Mich.) to remove bacteria and insoluble DHP. A 0.4-ml portion of the filtrate was counted in 10 ml of liquid scintillation cocktail. The pH of the filtrate was adjusted to 2.5 to 3 with 10 M HCl. The radioactivity in the filtrate was counted as described above, and neutralized with 10 M NaOH (53). The radioactivity in the filtrate was counted as described above.

Nucleotide sequence accession numbers. The 16S rDNA sequences of strain E-37T and PCR products 37SW-1 and 37SW-2 have been deposited in the GenBank database under accession nos. U58356, U58354, and U58355, respectively. The accession numbers for the sequences of the organisms used to construct phylogenetic trees are as follows: Antarctic gas-vascular bacterium (23), 1U4583; dimethylubonopropionate (DMP)-degrading bacterium, L15345; E. coli, J01089; Erithrobacter sp. strain OCH 114, M59063; methanesulfonate-degrading bacterium, U62894; Paracoccus denitrificans, X69159; Photorhabdus luminescens gall symbiont, U37762; Rhodobacter capsulatus, D16427; Rhodobacter sphaeroides, D16424; Roseobacter aphelsa, X78313; Roseobacter denitrificans, X69159; and Roseobacter luminiferus, X78312.

RESULTS

Isolation. Isolate E-37T was obtained from a single colony on YTSS agar. After more than five transfers on the same medium, only one type of colony was observed on plates and one type of cell was observed in wet mounts. Thus, the culture was judged to be pure.

Cellular and colonial morphology. Light microscopic examination revealed that the cells were straight rods which stained gram negative and occurred singly. The cells did not form spores. The cells tended to form rosettes and aggregates, especially during the stationary phase, when large clumps were visible. After 1 week, colonies on complex or defined media were 0.5 mm or less in diameter, circular, convex with entire margins, and light cream colored.

An electron microscopic analysis showed that the cells of strain E-37T were straight rods that were approximately 2.3 μm long and 0.9 μm in diameter. Each cell exhibited polarity; the width of one-half of the cell was greater than the width of the other half (Fig. 1A). A holdfast structure was present at the thicker cell pole. Blank and vesicles were also observed on the cell surfaces and free in suspension. The cells were also covered by a dense network of fine fibrils that may have been polysaccharides. This capsular material was also observed by phase-contrast microscopy following negatively staining with India ink (38). Flagella were not seen attached to cells but were seen in suspension. However, the cells were not motile during exponential growth in marine broth 2216, on marine agar, or in BM containing succinate. The cells were not heat resistant, and endospores were not observed when cultures were examined by electron or light microscopy.

Culture and growth conditions. Isolate E-37T did not produce diffusible pigments on marine agar 2216 or BM containing yeast extract. It grew in Shioi’s marine medium, as modified by Shiba (45), but did not grow on tryptic soy agar. The best growth occurred in marine broth 2216 or BM containing Casamino Acids. Strain E-37T had an absolute requirement for NaCl and failed to grow when NaCl was replaced with KCl or LiCl. Cells lysed in distilled water if they were first washed with 0.5 M NaCl. Washing with 0.05 M MgCl2 prevented lysis (29, 40).

Growth in BM containing glucose, acetate, citrate, or glutamate without either a vitamin solution or 0.005% yeast extract was slow. Growth was enhanced by the addition of a combination of biotin, pantothenic acid, cyanocobalamin, and thiamine, nicotinic acid, pantothenic acid, and aminobenzoic acid. When each of these vitamins was added singly, growth was not stimulated. The addition of a combination of thiamine, nicotinic acid, pantothenic acid, and biotin had no effect. In the presence of vitamins or 0.005% yeast extract, growth was enhanced by vigorous shaking.

Similar growth rates and final cell densities were obtained in BM containing yeast extract and 0.1X, 0.25X, 0.5X, and 1X sea salts. The temperature range for growth was 4 to 41°C. Good growth occurred at temperatures from 10 to 41°C, and the optimum temperature was 30°C (Fig. 2). At 4°C, the growth rate was very slow, about 1.5 day-1. Isolate E-37T grew in BM containing glucose at pH values from 5.5 to 8.5, but did not grow at pH 9.5 (data not shown). The best growth occurred at pH 7.5.

Physiological and biochemical characteristics. Strain E-37T was catalase and oxidase positive. It did not form acid from glucose, did not reduce nitrite, and failed to grow under anaerobic conditions on marine agar 2216. It did not grow on plates containing BM supplemented with glucose without a source of fixed nitrogen. Although KNO3 or Casamino Acids could substitute for NH4Cl, considerably better growth was obtained with NH4Cl as the nitrogen source. Good growth was also obtained when l-tryptophan was the sole carbon and nitrogen source. In the presence of NH4Cl, the following compounds supported growth: glucose, fructose, mannose, cellobiose, xylose, glycerol, pyruvate, formate, acetate, propionate, butyrate, succinate, malate, DL-β-hydroxybutyrate, citrate, methanol, ethanol, 2-propanol, l-butanol, l-alanine, l-arginine, l-aspartate, l-asparagine, l-glutamate, l-glutamine, l-histidine, l-leucine, l-phenylalanine, l-proline, l-serine, l-tryptophan, N-acetyl-l-glucosamine, benzoate, p-hydroxybenzoate, p-coumarate, cinnamate, ferulate, and vanillate. The
FIG. 1. Ultrastructure of isolate E-37T as revealed by transmission electron microscopy of negatively stained samples. (a) Cell covered by polysaccharide fibrils (P) aggregated into bundles. The cell exhibits polarity; the right half has a greater diameter than the left half and has a holdfast structure (H) at the cell pole; blebs (B) and vesicles (V) are close to the cell surface. Bar = 0.2 μm. (b) Polysaccharide fibrils attached to the cell surface and the fibrillar components of the holdfast structure (FH). CM, cytoplasmic membrane. Bar = 0.1 μm. (c) Dense network of polysaccharide fibrils and vesicles in the neighborhood of the cell surface. Bar = 0.1 μm. (d) Preparation showing detached flagellum (FL) and cell debris. Bar = 0.1 μm.
Substances that were not utilized for growth included sucrose, rhamnose, lactose, 1-propanol, methylamine, benzene, tolue-ene, phenol, salicylate, glycine, l-isoleucine, l-lysine, l-methi- onine, l-threonine, and l-valine. Isolate E-37T did not hydro- lyze Tween 80, chitin, gelatin, starch, birchwood xylan, or agar.

During growth on aromatic compounds, cultures of E-37T decreased the UV absorbance of the supernatant to the point when E-37T was grown in BM containing 2 mM acetate and in the presence of Na$_2$SO$_4$, up to a concentration of 20 mM. However, the cell yields, as determined from the G+C content of DNA. The guanine-plus-cytosine content of strain E-37T DNA was 65.0 ± 0.2 mol% (mean ± standard deviation; n = 6).

Major cellular fatty acids. Strain E-37T contained the following major fatty acids: 16:0, 8.6%; 18:0, 6.8%; 12:1 30H, 3.6%; and 19:0 cyclo 08c, 1.8%. This bacterium also contained the following additional major fatty acids that were not quantitated due to poor resolution of the chromatography system: 18:1 a7c, 18:1 a9t, and 18:1 a12t.

Molecular phylogenetic analysis. The sequence of approximately 1,400 bp of the 16S rRNA gene, corresponding to 97% of the 16S rRNA gene, was obtained. A parsimony and neighbor-joining analysis performed with representatives of the eu- bacterial and archaeabacterial groups placed E-37T in the α subclass of the Proteobacteria, close to the genus Roseobacter (levels of similarity, 93 to 94% [30, 43, 44]). The closely related sequences included the sequences of Roseobacter algicola (30), the heterotrophic sulfite oxidizer Sulfitobacter pontiacus (47, 48), a DMSP-degrading bacterium (32), a methanesulfonate-degrading bacterium (51), an Antarctic gas-vacuolate bacterium (23), and the red alga Prioritis lanceolata gall symbiont, all of which were isolated from marine environments. E-37T was most closely related to Sulfitobacter pontiacus (level of similarity, 95%). The levels of relatedness to other members of the α-3 subclass of the Proteobacteria were somewhat lower; the levels of similarity to Paracoccus spp. were 91 to 92%, and the levels of similarity to Rhodobacter spp. were less than 90% (Fig. 3 and data not shown).

Bootstrap analysis performed with the neighbor-joining and parsimony analysis revealed an unambiguous affiliation with the α-3 subclass and that strain E-37T was more closely related to Roseobacter denitrificans and Roseobacter litoralis than to the genus Paracoccus or the genus Rhodobacter. The bootstrap values were 100% in both cases for the branches that clustered E-37T with Roseobacter denitrificans and Roseobacter litoralis (data not shown). When other sequences in the group were considered, the position of E-37T with respect to Sulfitobacter pontiacus, the Antarctic gas-vacuolate bacterium, and Roseobacter algicola had low bootstrap values when the neighbor-joining and parsimony methods were used. The method of analysis used resulted in different topologies and different positions for E-37T with respect to these three sequences. Regardless of the method and sequences used, E-37T was always placed close to the group containing Roseobacter denitrificans, Roseobacter litoralis, and the Prioritis lanceolata symbiont. The association of E-37T with Sulfitobacter pontiacus was not robust since the method used and the different ingroups included in the analyses yielded different topologies. When the related sequences retrieved from seawater were included in the analyses, the bootstrap values were greater than 85% for the association of E-37T with 37SW-1 and for the association of E-37T with 37SW-2, whereas the sequence of Sulfitobacter pontiacus

![FIG. 2. Growth response of strain E-37T to temperature in BM containing yeast extract.](image-url)

FIG. 3. Phylogenetic position of strain E-37T based on its 16S rRNA sequence. Representatives of the most closely related group in the α subclass of the Proteobacteria are included for comparison. The dendrogram was constructed by using the results of an analysis of approximately 1,300 bp of the sequence, corresponding to unambiguous positions in all of the sequences used. The tree was generated by using the neighbor-joining method contained in the PHYLIP phylogeny inference package. The numbers are the bootstrap values for each branch; the numbers in parentheses are parsimony bootstrap values (only values greater than 50% are shown).
did not exhibit any association with these three sequences (data not shown).

A 16S rRNA secondary structure in the region from position 180 to position 220 is characteristic of the α subclass of the Proteobacteria (55) (A) and predicted secondary structure of the corresponding region of E-37T 16S rRNA (B). The shaded region indicates a deletion in the 16S rRNA of E-37T. Substitutions between strain E-37T and sequences 37SW-1 and 37SW-2 are indicated. There may be other differences in the region from position 138 to position 155 (which was covered by the upstream PCR primer). The circled base is a conserved position in the α subclass that is different in E-37T, 37SW-1, and 37SW-2. The numbers indicate base positions in E. coli. The helix numbers are the helix numbers used by Dams et al. (12).

**FIG. 4.** Secondary structure of helix 11 of the 16S rRNA of the α subclass of the *Proteobacteria* (55) (A) and predicted secondary structure of the corresponding region of E-37T 16S rRNA (B). The shaded region indicates a deletion in the 16S rRNA of E-37T. Substitutions between strain E-37T and sequences 37SW-1 and 37SW-2 are indicated. There may be other differences in the region from position 138 to position 155 (which was covered by the upstream PCR primer). The circled base is a conserved position in the α subclass that is different in E-37T, 37SW-1, and 37SW-2. The numbers indicate base positions in *E. coli*. The helix numbers are the helix numbers used by Dams et al. (12).

The range of catabolic activities of strain E-37T is consistent with the numerical abundance of this organism in the pulp mill waste enrichment culture examined. The isolate was able to utilize cellulose and transform lignin, natural polymers that were present in the enrichment culture and in the salt marsh system from which the inoculum was obtained (4, 24). Strain E-37T was also able to grow on lignin-related compounds, such as *p*-coumarate, cinnamate, ferulate, and vanillate. Methanol and other C₆ compounds were also utilized, a characteristic of other bacterial isolates capable of growing on pulp and paper industry wastes (18, 21).

Although the rate of mineralization of DHP was limited (only 3.5% after 1 month), the rate of solubilization was significant (54% after 1 month). Greater solubilization than mineralization has been reported for other lignin-degrading bacteria; many actinomycetes, for example, release soluble lignin compounds during degradation of lignocellulose (10). Other nonfilamentous bacteria are able to solubilize lignin prepara-
Isolate E-37\textsuperscript{T} is a marine bacterium that is gram negative, rod shaped, and strictly aerobic. As determined by 16S rRNA analysis, this organism was unambiguously affiliated with a group of marine bacteria in the \(\alpha-3\) subclass of the Proteobacteria. Its G+C content, absence of bacteriochlorophyll \(a\), and major fatty acid profile exclude strain E-37\textsuperscript{T} from Roseobacter denitrificans or Roseobacter litoralis (45). The most similar 16S rRNA sequence was that of Sulfitobacter pontiacus (level of similarity, 95\%). However, E-37\textsuperscript{T} did not oxidize sulfite with ferricyanide as an artificial electron acceptor, a property that is characteristic of the genus Sulfitobacter (47, 48). In addition, Sulfitobacter-pontiacus did not have the deletion in the region from position 180 to position 220 present in the E-37\textsuperscript{T} sequence. Sufficient phenotypic differences from all of the previously described species belonging to the \(\alpha-3\) group were found to support assignment of E-37\textsuperscript{T} to a new taxon.

Even though the 16S rRNA analysis revealed that strain E-37\textsuperscript{T} is phylogenetically affiliated with the \(\alpha\) subclass, a novel secondary structure in the region of the 16S rRNA molecule from position 180 to position 220 is proposed. This region is widely conserved elsewhere in the \(\alpha\) subclass, so the novel structure found in E-37\textsuperscript{T} may be diagnostic for the new taxon. The novel secondary structure is characteristic of strain E-37\textsuperscript{T} and two uncultured organisms that were detected by PCR amplification of DNA extracted from coastal seawater. Thus, a signature secondary structure for this region is proposed based on the similarity of the secondary structures of the three sequences.

**Description of Sagittula gen. nov.** Sagittula (Sa.git’tu.la. L. fem. n. Sagittula, small arrow, referring to the shape of the bacterium). Cells are rod shaped, gram negative, strictly aerobic, and oxidase and catalase positive. Each cell has a holdfast structure, and the cell envelope has numerous surface vesicles derived from the outer membrane. Cells form rosettes and aggregates and grow on sugars, fatty acids, and amino acids. Sea salt-based medium is required for growth. The type species is Sagittula stellata.

**Description of Sagittula stellata sp. nov.** Sagittula stellata (stel.la’ta. L. adj. stellata, starry). The cells of type strain E-37\textsuperscript{T} are rod shaped (approximately 2.3 \(\mu\)m long, and 0.9 \(\mu\)m in diameter) and have numerous vesicles on their surfaces. Colonies on marine agar 2216 are light cream colored.

The temperature range for growth is 10 to 41\(^\circ\)C, and optimal growth occurs at 30\(^\circ\)C. The optimal pH is 7.5, and the pH range is 5.5 to 8.5. The organism is a strict aerobe and does not denitrify. It accumulates polyhydroxybutyrate. It utilizes various carbohydrates and amino acids and some aromatic compounds, such as \(p\)-coumarate, cinnamate, ferulate, and vanillate, and it exhibits oxidase, catalase, and cellulase activities. During growth on glucose, cells are able to partially transform synthetic lignin. Growth without vitamins or yeast extract is slow. Capsules are produced in liquid medium.

Cells are susceptible to ampicillin, chloramphenicol, kanamycin, penicillin G, streptomycin, tetracycline, and vancomycin.

The guanine-plus-cytosine content of the DNA as determined by high-performance liquid chromatography is 65.0 mol\%. On the basis of its 16S rRNA sequence, E-37\textsuperscript{T} belongs to the \(\alpha\) subclass of the Proteobacteria and is related to Sulfitobacter pontiacus and Roseobacter spp. The organism was isolated from a marine enrichment community that was growing on pulp mill effluent as the sole carbon source and was originally inoculated with seawater from a salt marsh on the coast of Georgia. Type strain E-37\textsuperscript{T} has been deposited in the American Type Culture Collection as strain ATCC 700073.
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